

CLAIM LISTING

The listing of claims will replace all prior listings of claims in the application:

Claims 1-15 (cancelled).

16. (previously presented) A recombinant nucleic acid molecule encoding a modified pneumolysin polypeptide comprising one or more amino acid substitutions in a wild-type pneumolysin polypeptide having the amino acid sequence of SEQ ID NO:3, wherein said one amino acid substitution occurs at a position selected from the group consisting of position 61, 148, and 195 of SEQ ID NO:3, or wherein said more than one amino acid substitutions occur at positions selected from the group consisting of 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257 of SEQ ID NO:3, and wherein said modified pneumolysin polypeptide is soluble, elicits antibodies which are cross-reactive with wild-type pneumolysin, and has attenuated hemolytic activity.
17. (currently amended) A recombinant nucleic acid molecule, ~~comprising the pneumolysin nucleic acid sequence of SEQ ID NO: 1 or~~ wherein the recombinant nucleic acid molecule is a variant of the nucleic acid sequence of SEQ ID NO: 1, said variant encoding a modified pneumolysin polypeptide comprising an amino acid sequence of SEQ ID NO:3 having one amino acid substitution or a group of amino acid substitutions selected from the group consisting of:
- K17→R, K18→N, S61→P, N66→Y and I101→T;
- D41→G, T172→A, F195→I and K255→G;
- T63→S, V127→E, N128→H and M148→K;
- I33→T, I46→T, L83→S, S239→R and D257→G;

V45→A, D102→G, Q189→R and F195→V;

F195→V;

F195→I;

M148→K; and

S61→P

and wherein said modified pneumolysin polypeptide is soluble, elicits antibodies which are cross-reactive with wild-type pneumolysin, and has attenuated hemolytic activity.

18. (previously presented) The molecule of claim 16 or claim 17 as contained in a vector.
19. (previously presented) A microorganism or cell that has been transformed to comprise the recombinant nucleic acid molecule of any of claims 16-17 or claims 32-40.
20. (previously amended) The microorganism or cell according to claim 19, wherein the microorganism is selected from the group consisting of bacteria and yeast, and the cell is selected from the group consisting of mammalian cells and insect cells.
21. (previously presented) The microorganism according to claim 20, wherein the microorganism is *E. coli*.

Claims 22-26 (cancelled).

27. (withdrawn) A method for killing bacteria comprising contacting said bacteria with antibodies to an immunogenic molecule comprising a modified pneumolysin comprising one or more amino acid substitutions in a wild-type pneumolysin polypeptide having the amino acid sequence of SEQ ID NO:3, wherein said one amino acid substitution occurs at a position selected from the group consisting of position 61, 148, and 195, or wherein said more than one amino acid substitution occurs at positions selected from the group consisting of 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257, and wherein said modified pneumolysin polypeptide is soluble, elicits antibodies which are cross-reactive with wild-type pneumolysin, and has attenuated hemolytic activity in the presence of complement.
28. (withdrawn) The method according to claim 27, wherein the immunogenic molecule is a polysaccharide-polypeptide conjugate wherein the polysaccharide is a bacterial capsular polysaccharide.
29. (withdrawn) A method for immunization of mammals comprising administering a vaccine comprising the modified pneumolysin polypeptide comprising one or more amino acid substitutions in a wild-type pneumolysin polypeptide having the amino acid sequence of SEQ ID NO:3, wherein said one amino acid substitution occurs at a position selected from the group consisting of position 61, 148, and 195, or wherein said more than one amino acid substitution occurs at positions selected from the group consisting of 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257, and wherein said modified pneumolysin polypeptide is soluble, elicits antibodies

which are cross-reactive with wild-type pneumolysin, and has attenuated hemolytic activity and a pharmaceutically acceptable carrier to said mammals.

30. (withdrawn) A method for obtaining modified pneumolysin polypeptides, wherein said modified pneumolysin polypeptides have reduced hemolytic activity and are suitable for eliciting an immunogenetic response which is cross-reactive with wild-type pneumolysin comprising the steps of:

(a) mutating a nucleic acid molecule encoding wild-type pneumolysin to produce mutated nucleic acid molecules encoding modified pneumolysin polypeptides, wherein the modified pneumolysin polypeptides comprise one or more amino acid substitutions in a wild-type pneumolysin polypeptide having the amino acid sequence of SEQ ID NO:3, wherein said one amino acid substitution occurs at a position selected from the group consisting of position 61, 148, and 195, or wherein said more than one amino acid substitution occurs at positions selected from the group consisting of 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257 and expressing the mutated nucleic acid molecules in host cells;

(b) assaying the modified polypeptide expressed by the host cells for hemolytic activity; and

(c) identifying the modified pneumolysin polypeptides having substantially similar molecular weight as native wild-type pneumolysin and which are refoldable.

31. (previously presented) The molecule of claim 16 or claim 17, wherein the molecule is incorporated into a vector selected from the group consisting of a plasmid, cosmid, bacteriophage and yeast artificial chromosome.
32. (previously presented) A recombinant nucleic acid molecule encoding the modified pneumolysin polypeptide pNVJ1.
33. (previously presented) A recombinant nucleic acid molecule encoding the modified pneumolysin polypeptide pNVJ20.
34. (previously presented) A recombinant nucleic acid molecule encoding the modified pneumolysin polypeptide pNVJ22.
35. (previously presented) A recombinant nucleic acid molecule encoding the modified pneumolysin polypeptide pNVJ45.
36. (previously presented) A recombinant nucleic acid molecule encoding the modified pneumolysin polypeptide pNVJ56.
37. (previously presented) A recombinant nucleic acid molecule encoding the modified pneumolysin polypeptide pNV103.

38. (previously presented) A recombinant nucleic acid molecule encoding the modified pneumolysin polypeptide pNV207.
39. (previously presented) A recombinant nucleic acid molecule encoding the modified pneumolysin polypeptide pNV111.
40. (previously presented) A recombinant nucleic acid molecule encoding the modified pneumolysin polypeptide pNV211.